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OBSERVATIONS AND EXPERIMENTS

ON

LIVING ORGANISMS IN HEATED WATER.

BY

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OBSERVATIONS AND EXPERIMENTS ON LIVING ORGANISMS IN HEATED WATER.

IN a former number of this Journal (vol. xxxiv, July, 1862), an account was given of some experiments on the formation of Infusoria in boiled solutions of organic matter, the result of which was, that such solutions, exposed only to air which had passed through iron tubes heated to a redness, became the seat of infusorial life. The same result followed when similar solutions were enclosed in hermetically sealed flasks, and subsequently exposed to the action of boiling water. In a few instances Infusoria appeared when the temperature was raised above 212° F. All living beings found under the above circumstances have been attributed either, 1st, to organisms or the germs of them, supposed to be contained in the fluid experimented with, or the air included in the flasks; or 2d, to the direct transformation of organic matter into new living beings, independently of any germs or living organisms whatever; or in other words to "spontaneous generation."

Abundant proof has been brought forward to show that the spores or germs of infusoria exist in the air in quantities amply sufficient to account for the presence of living organisms in solutions freely exposed.

There can therefore be no certainty of the existence of spontaneous generation in a given solution, until it can be shown, that this has been freed of all living organisms which it contained at the beginning of the experiment, and kept free of all such from without during the progress of it. On the other hand, this kind of generation becomes probable, whenever it is made certain that Infusoria do appear in solutions, in which the conditions just mentioned have been complied with.

We say probable, because their appearance under such circumstances would not amount to a proof. The absolute proof of spontaneous generation must come from the formation of living organisms out of *inorganic* matter. If infusoria are generated in solutions of organic matter, independently of spores or germs, the question may be fairly raised whether we do not begin the experiment with materials in which life already exists, even though this material is not in the form of distinct organisms.

The issue between the advocates and the opponents of the doctrine in question, clearly turns on the extent to which it can be proved that living beings resist the action of water at a high temperature, or on what Pouchet calls "vital resistance," for in nearly all the observations hitherto made, heated water has been the agent for the destruction of infusorial life preliminary to the beginning of an experiment.

The observations and experiments contained in this communication, have not been brought together either for sustaining or refuting the doctrine just referred to, but partly with the view of testing the accuracy of the experiments formerly made, and chiefly for the purpose of determining how far the life of certain kinds of low organisims is either sustained or destroyed in water which has been raised to a high temperature, a result which must be reached before spontaneous generation can be either asserted or denied. The evidence which will be adduced is derived from the following sources:

1st, from the phenomena of hot springs;

2d, from the appearance or non-appearance of Infusoria in solutions boiled for different periods of time, and exposed only to pure air;

3d, from the observed action of heat on the living organisms which the solution experimented with, was known to contain.

I.

Thermal Springs.—The study of organisms living in thermal waters is of great importance in connection with the investigation of the limit of vital resistance. Having become adapted, through a long series of years, to their surroundings, such organisms may be supposed to live under circumstances the most favorable possible, for sustaining life at a high temperature. It is a well known physiological fact that living beings may be slowly transferred to new and widely different conditions without injury; but if the same change is suddenly made they perish. In the experiments made in our laboratories, the change of conditions is relatively violent and therefore liable to destroy life by its suddeness, when otherwise it might possibly have been sustained. In the thermal waters therefore which give us a range of temperature as high as 212° F., we are more likely to find the highest degree of heat consistent with the maintenance of life than in our flasks. The following accounts from different observers have been selected as giving the highest temperature at which life has been noticed in hot springs. It must not be overlooked, however, that these have been thus far very imperfectly studied, especially with regard to the lowest organisms, and that we are by no means sure that the extreme limit of endurance in them has been ascertained.

Hot Springs of Luzon.—These springs were examined by Sonnerat* and his account of them is often quoted as evidence that they are inhabited by fishes and plants at a temperature of 187° F.† This assertion is not sustained by those who have visited them since Sonnerat. Dr. Abel who accompanied Lord Amherst in his mission to China examined them, and "saw no fish, but a small snake and a frog which were not only dead but boiled."‡ Marion de Procé, who has examined the same springs with care states that the waters where the fish were actually found did not exceed 36° C. (or 96·8° F.)‡ Prof. James D. Dana, one of the naturalists of the U. S. Exploring Expedition also visited them; he makes no mention of animal life, but gives the important observation "that the stones were covered with a white incrustation which appears to be siliceous, and a species of feathery vegetation occurs also upon those bordering the streamlets where the temperature is 160° F., and presenting various shades of green and white."§

Hot springs of the Ouachita.—These are situated in Hot-spring Co., Arkansas, and the four hottest are stated by Major Long to have the temperature of 132°, 140°, 148° and 150° F., respectively. He says "not only Confervæ and other vegetables grow in and about the hottest of them, but great numbers of little insects are constantly seen about the bottoms and sides." "A small bivalve testaceous animal adheres to the plants and lives in such a high temperature."‖

Hot springs of Sorujkund.—Dr. Joseph D. Hooker found in these springs *Confervæ* which Berkeley referred to the genus *Leptothrix* "growing on the margins of the tanks and in the hottest water; the brown is the best salamander, and forms a belt in deeper water than the green; both appear in luxuriant strata, wherever the temperature has cooled down to 168° F., and as low as 90°."**

The hot springs of Pugha, in Thibet reach the temperature of 174° F., and *Confervæ* and *Oscillatoriæ* were found growing in them by Capt. Strachey.††

Hot springs of Mariara and La Trinchera.—Humboldt has given the result of his own carefully made observations on the thermal waters of South America, among the most remarkable of which are those of Mariara and La Trinchera, and of these the latter is the hottest. Of La Trinchera he says, "We were

* Journal de Physique, Avril, 1774, t. iii, p. 256.

† Spallanzani Opuscoli, Milano, 1826, p. 69.

‡ Edwards, Influence of Physical Agents on Life. Translated by Dr. Hodgkin and Dr. Fisher. London, 1832, p. 466.

§ Geology of the U. S. Exploring Expedition, p. 543.

‖ Long's Expedition to the Rocky Mountains, vol. ii, p. 291. Philadelphia, 1822.

** Himalaya Journals, vol. i, p. 27. London, 1854.

†† Ibid, vol. ii, p. 379.

surprised at the luxuriant vegetation that surrounds the basin; *Mimosas* with slender pinnate leaves, *Clusias* and fig trees have pushed their roots into the bottom of a pool, the temperature of which was 85° C. (185° F.), and the branches of these trees extend over the surface of the water at two or three inches distant." "An *Arum* with a woody stem, and with large sagittate leaves, rose in the very middle of the pool, the temperature of which was 70° C. (158° F.) Plants of the same species vegetate in other parts of those mountains at the brink of torrents, the temperature of which is not 18° C. (64·4° F.)*

Hot springs of California.—I am indebted to Dr. William H. Brewer, Botanist to the Geological Survey of California, and to Mr. William T. Brigham of Boston for the information which follows, in relation to the thermal waters of this state which they have personally examined. Dr. Brewer's observations as will be seen by the following extract from his letter to me, show the existence of plants in water of a considerably higher temperature than even that of La Trinchera. He says, "the place where the greatest temperature was noticed, in which plants occur is the Geysers, in Lake (formerly Sonoma) county about seventy-five miles north of San Francisco. Vegetable forms flourished in these waters at various temperatures up to 93° C., (199·6° F.,) but were most abundant in waters of the temperatures of 52° to 55° C. (125°-131° F.)"

"At the higher temperature they were not abundant and existed as grains like Nostoc or Protococcus, intensely green and rather dark. They were observed in several cases where the water was above 90° C., but were more common in the streams, as the water cooled, and formed large slimy masses. Much of these were in long slender filaments like Confervæ."†

"The temperatures given here were carefully observed with a standard centigrade thermometer, with a naked elongated bulb."

Mr. Brigham visited the same springs and made a collection of some of the Confervoid plants growing in them. In regard to animal life he says, "although I looked with great care I could find no living animals in the water, but boiled insects were rather common. These were not fair cases, as the waters were so strongly impregnated with sulphur and acid that these alone might account for the absence of animal life. On the bank where the temperature ranged from 197° to 207° F., spiders were abundant and seemed to feel no inconvenience from the heat. I saw several feeding on the bodies of insects boiled by the water. The spiders were seen even standing on the water which

* Personal Narrative, Bohn's edition, London, 1852, vol. ii, p. 38.

† Since the above was written, Prof. Dana and Dr. Brewer have published in this Journal, 1866, vol. xli, p. 389, various observations and remarks in relation to life in hot springs and in high temperatures.

was 176° F.; their bodies were not immersed and the hairs on the legs might, as is often the case, repel the water."

Hot springs of Iceland.—"Mr. Flourens exhibited to the Academy, Confervæ collected in Iceland by M. Descloizeaux who found them growing in the Gröf at a temperature of 98° C., or 208° F."*

Many other accounts† of living organisms in thermal waters might be given, but they relate mostly to a temperature lower than that of those already mentioned. Our object has been to bring together only the hottest, and with regard to which the evidence is trustworthy.

The statements we have quoted give satisfactory proof that different kinds of plants may live in water of various temperatures, as high as 168° F., as observed by Dr. Hooker in Sorujkund, 174° as observed by Capt. Strachey in Thibet, 185° as observed by Humboldt in La Trinchera, 199° as observed by Dr. Brewer in California, and 208° as observed by Descloizeaux in Iceland. The lowest forms only are found in the hottest waters.

With regard to animals the testimony is much less complete. The highest temperature at which they have been found, in so far as we have seen any evidence was, as stated by Major Long, 150° F., in the hot springs of the Ouachita where he found "bivalve testaceous animals" and insects. In the instance mentioned by Mr. Brigham, although the spiders were in air the temperature of which was from 197° to 207° F., it is doubtful whether their bodies were as hot. They are air-breathing animals, but it is not unlikely that the heat of their bodies is kept down as in others whose breathing is aerial by the evaporation from their respiratory and other surfaces. Otherwise it does not appear how the albuminous matter in their fluids is kept from coagulation. When walking on the surface of the water it is most probable, as Mr. Brigham suggests, that their feet are not immersed, but that these, as in Hydrometr~~o~~, repel the water; the insects therefore might be wholly surrounded by air, in which case the temperature would be lower than that of the water. If a thermometer be held a short distance, not exceeding a half-inch, from the surface of boiling water, the temperature indicated will be many degrees below the boiling point. A marked difference exists even when the bulb and water nearly touch.

To show the importance of great accuracy in determining the temperature at the precise spot where the organisms are found we will cite the following statement, where a very erroneous result would have been obtained if the temperature of the sur-

* Comptes Rendus de l'Academie des Sciences, xxiii, 1846, p. 934.

† See Edwards, Influence of Physical Agents on Life, London, 1832, p. 407. Carpenter, Gen. and Comp. Physiology, Philadelphia, 1851, pp. 57 and 70.

face-water had been taken as representing that of the whole mass. Tripier in his account of the baths of Hamman-mes-Koutin, in Algeria, saw fishes in water the surface of which had a temperature of 56° C. (132° F.), but in the lower layers from which the fishes did not rise, the thermometer indicated only 40° C. (104° F.) It is to be feared that this possible difference of temperature has not always been kept in view, by those who have reported the existence of animals in water of a high degree of heat.

Dr. Carpenter in his General and Comparative Physiology mentions several instances of animals living in a high temperature and also states, that "at the island of New Amsterdam there is a mud spring, which, though hotter than boiling water, gives birth to a species of Liverwort."* It is presumed that this is the island of the same name referred to by Humboldt, but he only states that the springs in question were much hotter than those of the Mariara, which last were from 132° to 135° F.† It has also been asserted that Humboldt saw living fishes thrown out from the crater of Chimborazo at 210° F., and that they have been discharged at a higher temperature from the Geysers of Iceland.‡ In the first instance it was at Cotopaxi and not Chimborazo, that Humboldt made his observations. A species called *preñadillas* by the natives (*Pimelodus cyclopum*) is expelled from time to time from the crater, or clefts in the sides of it, in immense numbers; but he expressly states that the water expelled at the same time is not hot but cold and that the fishes are not so disfigured as to indicate that they had been exposed to a high temperature.§ We have not been able to find authority for the statement with regard to the Geysers.

II.

Experiments with boiled solutions of organic matter in sealed flasks.—These experiments, which are divided into two series, were made for the purpose of ascertaining to what extent certain kinds of organic solutions became the seat of infusorial life, notwithstanding a more or less prolonged exposure to boiling water, and the precautions taken to exclude the entrance of infusoria from without. They are in part repetitions of some of those formerly described, the results of which they fully con-

* Gen. and Comp. Physiol., 3d edition, Philadelphia, 1851, pp. 57 and 70.

† Humboldt's Personal Narrative, London, 1852, vol. ii, p. 24.

‡ Bibliothèque Universelle de Geneve, tome xx, p. 204, 1839.

§ Humboldt, Recueil d'Observations de Zoologie et d'Anatomie Comparée, 4to. Paris, 1811, p. 21.

firm. Great pains have been taken to ensure accuracy in conducting, as well as in observing and recording them.*

Series A. The flasks used in this series had a capacity of about 800 c. c. and had a long and slender neck; this was drawn out, about an inch from the end to a size which could easily be melted in a flame and in some instances the stem of a clay pipe, with a calibre of 0·08 inch, and in others, a copper tube 0·18 inch in diameter and filled with fine wires, was cemented into the mouth. The fluid in the flasks was boiled and thus the contained air expelled and replaced by steam. After the boiling had been continued sufficiently long, the whole was then allowed to cool slowly, the air to reënter through the tube, which last was kept meanwhile at a red heat. After the flask was cold it was hermetically sealed, the heat of the tube being carefully kept up until this was accomplished. Thus a boiled organic solution was obtained, in contact only with air which had been purified by heat.

Exp. I.—The contents of the flask were a few grains of meat and sugar, and 20 c. c. of water boiled 25 minutes. An infusorial film was formed on the 5th day; the flask was opened on the 31st. Vibrios were found in large numbers.

Exp. II.—50 c. c. of beef-juice and water were boiled twenty minutes. On the 4th day a thick infusorial film was formed over the whole surface of the fluid; the flask was opened on the 6th day and found to contain Vibrios and Bacteriums.

Exp. III.—The flask contained a small piece of beef weighing about three grammes, and 50 c. c. of water, which were boiled 30 minutes. An infusorial film was formed on the 3d day, which sank to the bottom and was afterwards replaced by another.

The flask was opened on the 27th day; the contents had a nauseous odor but were not putrid. The muscular fasciculi had fallen to pieces and the fluid had become slimy and viscid. Immense numbers of Monads filled the solution. Some feathery crystals were noticed, but the most striking circumstance in this

* M. Milne Edwards in referring to the former series of experiments reported by the writer of this article and to others similar to them, questions their accuracy, since they do not agree with those of M. Pasteur, and thinks the difference in the results depended upon a defective mode of conducting the experiments. The process employed in some of them was identical with that employed by M. Pasteur himself, and in others, with that made use of by M. Milne Edwards in his one experiment (see his *Leçons sur la Physiologie et l'Anatomie Comparèe*, t. viii, pp. 260, 269.) When flasks, holding organic fluids and air, are hermetically sealed and boiled for from fifteen minutes to three or four hours, and infusoria make their appearance nevertheless, the experiment cannot be called a faulty one, so long as only these results are claimed. The recent experiments of Dr. G. W. Child of Oxford, England, and those reported in this communication are a sufficient answer to the criticisms of M. Edwards. The experiment of Pouchet recorded in his *Nouvelles Experiences*, Paris, 1864, p. 224, is conclusive as to possibility of Infusoria appearing in boiled solutions exposed to pure air.

as well as in some other instances in which muscle was used, was the fatty degeneration of the fibres which had taken place subsequently to the boiling. The fibres were found in many stages of change, some of them having their contents finely granular, striæ still seen, while others were filled with oil-globules, and had no traces of striæ left. One specimen was examined by Dr. Calvin Ellis who was unable to recognize any difference between the appearances which it presented and those of ordinary fatty degeneration of the muscles.

Exp. IV.—The flask contained very thin wheat paste mixed with saliva and water; the boiling was continued 25 minutes. No film was noticed until the 30th day, the flask was opened on the 68th day, and the fluid found filled with Monads.

Exp. V.—A few grains each of meat and sugar in 20 c. c. of water were boiled 25 minutes. An infusorial film appeared on the 4th day. The flask was opened on the 50th day by Prof. Henry James Clarke by whom the accompanying figures were carefully drawn from nature.

Fig. 1. *Vibrio baccillus*, enlarged two thousand diameters; these are very numerous and move quickly; the joints appear to be united by a gelatinous substance which is quite transparent and move on each other by a series of flexions and extensions; the number of segments is variable.

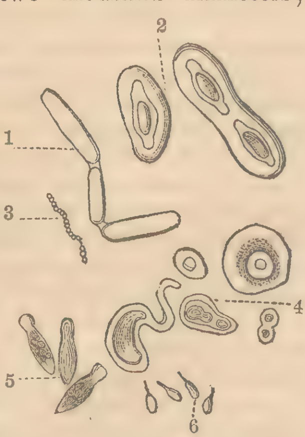

Fig. 2. *Bacterium;* enlarged four thousand diameters. Prof. Clarke has given especial attention to these bodies, and has shown that they are somewhat more complex than generally supposed. They consist of a dark colored oval nucleus, around which is a transparent space, and around this a gelatinous envelope of extreme delicacy. They are sometimes seen single but generally double; more than two are rarely united together.

Fig. 3. A linear series of globules enlarged five hundred diameters; these have the power of locomotion.

Fig. 4. Excessively faint and colorless bodies of great delicacy, and changing their form under the slightest pressure. They closely resemble the substance described by Virchow as *myeline* which not only forms the medullary sheath of the nerves, but is also found abundantly elsewhere. He says "there scarcely exists a tissue rich in cells in which this substance does not exist in large quantities."*

* Cellular Pathology, translated by Dr. Chance, p. 234. London, 1860.

Fig. 5. This figure, also drawn by Prof. Clarke, represents an organism commonly seen in nearly all of the experiments. It is enlarged thirty-five hundred diameters.

Fig. 6, represents the form of the bodies we have called Monads, and each has a ciliated appendage and is locomotive.

SERIES B.—The flasks in which the experiments described below were made, all had the same capacity, viz. about 70 c. c., and the quantity of solution used in each was from 5 to 8 c. c. After the fluid to be experimented with had been introduced they were sealed at the temperature of the room, placed in a covered vessel containing water, and the whole heated to 212° F., and boiled for various periods.

This method corresponds substantially with that of the Needham and Spallanzanni. It is in all respects more simple and easier than that in which the apparatus is complicated with a heated tube. This last beyond a question destroys all organisms contained in the air which enter the flask through it, but is without effect on such as may be contained in the solution, or adhere to the inner surface of the glass. These come in contact only with boiling water or steam, and unless destroyed by one or the other of these, would be sufficient to vitiate any experiment, however careful the adjustment and heating of the tube may have been. We therefore believe that the tube is an unnecessary and useless complication of the apparatus.

Exp. VI.—Six flasks prepared as above, and containing from 5 to 8 c. c. of beef juice and water were boiled 30 minutes. The albuminous matter was coagulated, but the fluid portion became perfectly transparent. The contents became turbid and an infusorial film was formed in four of the flasks on the 7th day, and a few days afterwards in the others.

Exp. VII.—Six flasks, each containing about 1 gram of beef and 5 c. c. of water were boiled 30 minutes. An infusorial film was formed on the surface of all of them on or before the 7th day.

Exp. VIII.—A single flask containing a few grains of finely ground bean flour and 5 c. c. of water was boiled 48 minutes. The mixture became filled with Vibrios on the 4th day.

Exp. IX.—Six flasks containing beef juice and water were boiled 30 minutes. A thick infusorial film was formed in all of them on or before the 7th day.

Exp. X.—Six flasks containing each a few milligrams of beef and 5 c. c. of water were boiled 30 minutes. An infusorial film was formed on all on or before the 17th day.

In the preceding experiments of this series, the boiling was continued thirty minutes in four, and forty-eight in the fifth. The following experiments were made for the purpose of ascertaining whether prolonged boiling would be attended with a different result.

Exp. XI.—Five flasks, each containing a few grains of beef, and about 5 c. c. of water were prepared as in the preceding experiments; one was boiled 32 minutes, one 50 minutes, and the other three 1 hour and 20 minutes each.

An infusorial film formed in the first on the 2d day, in the second on the 6th day, in one of those boiled 1 hour and 20 minutes on the 8th, and in the others on the 10th. All were examined and found to contain *Vibrios* and *Bacteriums;* and in addition the first and second contained the "myeloid substance" already described, fig 4. Those which were boiled longest were the latest in developing infusoria; but of those boiled 1 hour and 20 minutes, they appeared two days later than in the other.

Exp. XII.—Twenty-four flasks each containing about 5 c. c. of beef juice were divided into six series of four each, and boiled for different periods as indicated in the following table; this table also gives the day of the appearance of the infusoria in each of the flasks belonging to a given series.

Series.	Time boiled.		Day of appearance of Infusoria.			
I.	0^h	30^m	3	4	6	0
II.	0^h	45^m	4	4	4	4
III.	1^h	00	4	4	4	0
IV.	1^h	15^m	4	4	0	0
V.	1^h	30^m	4	0	0	0
VI.	2^h	00	0	0	0	0

Seven of the flasks produced no infusoria, and the number in which they did appear becomes less the longer the boiling was continued.

Exp. XIII.—Of seventeen flasks containing beef-juice eleven were boiled 45 minutes, and 6 were boiled 2 hours. Infusoria appeared in all of the first series except one, at the end of the second day, and in the remaining one of this series and in all of the second on the 3d day.

It will be seen at a glance that Expts. XII and XIII differ in their results, and that of four flasks in Ex. XII boiled 1 h. and 30 m., three produced no infusoria, while in Ex. XIII the infusoria appeared in all, six of which were boiled two hours. We have several times met with similar differences in successive experiments. Sometimes flasks boiled for a single hour became wholly inert, even when the number of them was quite large. We can only insist that in the above experiments the flasks were properly sealed, and that there was no error as to the time for which the boiling was kept up.

Exp. XIV.—Twenty flasks containing extract of beef were treated as follows:

	No. of flasks in each series.	Time boiled.	Day on which infusoria appeared.				
Series I.	5 flasks.	1^h	6	6	6	6	6
" II.	5 "	2^h	6	6	6	6	7
" III.	5 "	3^h	6	0	0	0	0
" IV.	5 "	4^h	0	0	0	0	0

No flask was opened until the sixth day, though there were slight indications of infusoria three days previously; actively moving Vibrios were found in large numbers. The experiment was discontinued on the 10th day, as there was no indication of further change.

Exp. XV.—Thirty-two flasks containing a boiled solution of "extract of beef"* were arranged in six series, and boiled for different times as seen in the following table:

	No. of flasks in each series.	Time boiled.	Day on which Infusoria appeared.				
Series I.	5	$0^h\ 30^m$	1	1	2	2	2
" II.	5	1 00	2	2	2	2	2
" III.	5	1 30	2	2	2	2	2
" IV.	5	2 20	1	2	2	2	2
" V.	5	3 30	2	2	3	3	?
" VI.	7	4 00	2	2	2	4	4
			4	4			

An infusorial film formed in all the flasks on the days indicated, except in the one marked doubtful in series V, and those of series VI, in which infusoria appeared on the 4th day. None of these last had a film, but, as in the one marked doubtful in series V, had an immense number of minute bodies, which formed a cloud when the flask was shaken, and were not there when the experiment was begun. These bodies were spherical, had an outer wall, and a nucleus which was also a hollow body. They were of different sizes, and seemed to be undergoing multiplication by division. Their real nature must be considered doubtful. The films in the other flasks consisted chiefly of Bacteriums.

The preceding experiments show that if the boiling of the flasks be continued for four hours, as in Exp. XV, the infusoria may appear nevertheless—though in other cases it has happened, as in Exp. XII, that life ceased to be manifested if it was continued only for two hours.

In pushing the experiments still further, we have not found that infusoria appeared in any instance if the boiling was prolonged to five or six hours. Several experiments, in which many flasks were used, were tried, but the result was uniformly the same. Thus a limit to the development of infusoria in boiling water was reached.

* This is Borden's concentrated juice of beef, evaporated to a nearly solid substance, is free from tissues, and is entirely soluble.

III.

Experiments to show the effect of boiling water on living infusoria. —In view of the results given above, viz., the development of infusoria in sealed flasks, notwithstanding the precautions taken, the question naturally arises whether the infusoria which appeared were already in the flask and resisted the action of the water at 212° F. If not, then the invasion of the contents of the flasks by them is easily accounted for, since experiment shows that even a very few individuals if present may multiply so rapidly as to fill and make turbid a given solution in a very short time. But on the other hand, if the infusoria perish during the boiling, then any subsequent appearance of them under the circumstances, must be explained in some other way than by the common process of reproduction.

Since large numbers of infusoria can be proved at all times to be floating in the air, we must assume that some are included in every flask, and since certain organisms live in thermal waters at a very high temperature, within a few degrees even of boiling water, the experimenter is called upon to show whether water at the boiling point is or is not destructive of life, before he can venture to offer a theory of the origin of the infusoria in the flasks. It should not be overlooked that a marked difference exists between the conditions of life in the flasks and the thermal springs; in the former, the temperature is suddenly raised from that of the air to the boiling point, while in the latter, the organisms inhabiting them have become adapted to their surroundings through long periods of time. Furthermore it must be remembered that in the two cases, we have to deal with widely different species. It therefore becomes necessary to determine by direct experiment on the species of infusoria found in the flasks what *their* powers of resistance are.

Before proceeding to give the result of the experiments we have made, bearing upon this question, we will notice some of the statements which are constantly urged in support of the opinion that infusoria are capable of resisting water of a very high temperature. Among these are the ones relating to the well known experiments of Doyére and others, in which Tardigrades and Rotifers are asserted to have resisted a heat of 248° F. In these cases the important condition that the organism was in a *dry* and not in a moist state is often overlooked. In truth Doyére himself expressly mentions that in a *moist* condition they perished at 122° F.*

* Annales des Sciences Naturelles, t. xviii, 1842, p. 29. The whole subject as regards the resistance of *dried* Rotifers to heat, was investigated in an exhaustive manner by a commission consisting of Balbiani, Berthelot, Brown-Sequard, Dareste, Guillermen and Robin. See Comptes Rendus et Memoires de la Societé de Biologie, 1860.

In the alleged instances of seeds resisting the action of boiling water, it may be reasonably doubted whether in these the results have not been misunderstood. Spallanzanni clearly proved that if the seeds experimented upon by him were soaked previously to the boiling, they did not germinate.* So long as the water does not penetrate to the germ, this is no more influenced by it than if the germ were exposed to dry air heated to the same degree, and this it easily resists. Water penetrates the seeds of many plants and especially of some of the *Leguminosæ* very slowly; in the case of those of *Gleiditchia* and *Laburnum*, we have found several days and even weeks necessary for the complete penetration of cold water, though when the water is hot it penetrates much more readily. If therefore the seeds are dry when immersed, and are boiled for a few minutes only, they may still germinate. If they are moistened beforehand, the action of boiling water has been found uniformly fatal. In one of our experiments twenty-eight seeds of *Gleiditchia* were soaked until their coverings became soft and swollen; one half were planted at once, and the others after having been boiled five minutes. None of the boiled ones germinated while all the others did. Similar experiments with beans and with several other kinds of seeds ended in a similar manner.

Pasteur has given the result of his own observations on the effects of high temperature on the spores of some of the different kinds of Cryptogams, and states that they resist when dry 248° to 257° F., but perish at 266°.† He gives no experiments in which the spores were exposed to heated water, or to steam. He excuses himself, however, on the ground of being unable to devise a sufficiently rigorous method of experimentation.‡ We believe the alleged difficulty can be met, and shall endeavor to show by a series of comparative experiments that definite results may be obtained. From the manner in which Pasteur states his own results, he gives the impression that in view of the fact that the spores resist when *dry* a temperature of 257° F., it is reasonable to suppose that they will resist 212° when *moist.* He does not assert this, but leaves his reader to infer it. We have tried many experiments upon different kinds of moulds and yeast plants and have found, as nearly all observers have, that they perish at 212° F. Every one is familiar with the process of "scalding" in domestic economy, which destroys the kinds of cryptogams ordinarily concerned in fermentation, and on the efficacy of which the preservation of canned meats and other articles depends. As regards moulds the following experiment is decisive. Take any number of cups of paste and sow them all freely with spores; put one half in a covered vessel

* Oposcoli, Milano, 1826, p. 63.

† Ann. des Sc. Nat., t. xvi, 1861, p. 81.

‡ Ibid, p. 85.

containing a small quantity of water; boil this for a few minutes, thus exposing the paste and spores to steam, and then set all the cups in similar conditions for light and heat; none of the spores in the series which has been steamed will germinate, while all others will.

Payen is quoted by many writers as asserting that the spores of *Oïdium aurantiacum* germinated after having been exposed to 248° F. Pasteur himself expresses the belief that in this case the temperature has been erroneously determined.* Payen's own statements are somewhat conflicting. In the one most commonly quoted he says that spores heated in ordinary dough to 120° C. (248° F.), may still germinate, but are so altered at 140° C. (284° F.) that they do not germinate.† In another and later statement he presents the matter quite differently. "The spores of *Oidium*," he says, "preserve their vegetative faculty in the soft part of the bread, (*la mie du pain*) the temperature of which does not exceed 100° C. during the baking;‡ while in the crust, the temperature which exceeds 200° C. (392° F.) destroyed the vitality of the *Oidium*."§ There are here two conditions of the bread recognized; the moist interior which destroys the spores at 212° F., and the dry exterior which does so at 392° F. Mention has also been made of the germination of seeds taken from the raspberry jam, as proof that these had resisted the action of boiling water. But in none of these cases have we seen any evidence adduced to show that they had really been boiled. We have not been able to find a single instance in which seeds or spores or infusoria, excepting only Vibrios, Bacteriums and Monads, thoroughly moistened before the experiment, have resisted the prolonged action of boiling water.‖

The organisms which we have most commonly met with in our experiments in flasks, in fact almost the only ones, when the boiling was prolonged, are *Vibrios*, *Bacteriums* and *Monads*. The first and second are without doubt plants allied to the *Algæ;* the nature of *Monads* is more obscure. They are all among the lowest of living organisms. Leaving out of consideration ciliated infusoria which perish more easily, our inquiry now is, *at what temperature, or after how long an exposure to the action of boiling water are the Vibrios, Bacteriums and Monads killed, and by what signs can we know that they are dead?*—This question is a difficult one to answer.

* Ann. des Sc. Nat., t. xvi, 1861, p. 81.

† Ann. de Chim. et de Phys., 3me serie, t. xxiv, 1848, p. 254.

‡ The baking in this case was for the purpose of destroying the spores in loaves in store, and not for those going into the oven for the first time.

§ Precis de Chim. Indust. Paris, 1859, t. ii, p. 150.

‖ The assertion of M. Coste, that encysted infusoria resist the action of boiling water, has been shown to be incorrect by the conclusive experiments of M. Victor Munier.

The usual signs of life manifested by the infusoria found in the flasks are the following: 1st, locomotion in nearly all of the species; 2d, growth and reproduction; 3d, their reaction on the surrounding fluid, producing fermentation or putrefaction. The second and third are so intimately associated that the presence of one is almost a certain indication of the other. If a clear organic solution ferments, or becomes turbid, it may be safely inferred that living infusoria are present; nevertheless the correctness of this inference must be tested with the microscope. The absence of either of the above signs *alone* cannot be considered as a proof of death, and under certain circumstances all *signs* of life may cease, but the infursoria may still be alive. If for example, they are developed in a sealed flask, as soon as the organic matter convertible into infusoria is exhausted their activity ceases, and they remain dormant for months; we have kept them in this way for a year; but if fresh material is supplied to them, they at once resume their activity. Inactivity *in the presence* of organic material suitable for nourishment, and of air at the ordinary temperature, added to the absence of the other signs of life, must be considered as the best indication of death.

1. *Arrest of motion.*—The temperature at which motion ceases was determined as follows: a wide-mouthed bottle containing infusoria was set in a water-bath, and a thermometer suspended with its bulb in the infusorial fluid. The whole was gradually heated, and drops of the fluid were examined from time to time with the microscope, until the motions of the infusoria ceased. The following table gives the result of the examination of fluids taken from several different sources. The movements of the Vibrios lasted longer than those of all other kinds, and the temperature given below is that at which the motions of these were arrested. The motion of all the ciliated infusoria stopped at less than 130° F.

Motion ceased	Experiments.					Average.
	I.	II.	III.	IV.	V.	
	°	°	°	°	°	° °
In infusoria from a macerating tub at	57·5	57·0	56·0	57·0	56·0	56·7 C. = 134·06 F.
In infusoria from another tub at	55·0	57·0	58·0	57·0	58·0	56·4 C. = 133·00 F.
In infusoria from beef juice at	55·0	54·0	55·0	54·0		54·5 C. = 130·00 F.
In infusoria from a source not stated at	57·0	55·0	56·0			56·0 C. = 132·90 F.

2. *Arrest of growth and reproduction.*—In the following experiments as a matter of convenience the temperature was always carried to the boiling point, though it by no means follows that this was in all cases necessary. The object was to determine by comparative experiments, whether the growth and reproduction of the infusoria and their reaction upon a given solution is arrested or modified by their having been exposed to boiling

water. The solution used in nearly all was beef juice,* obtained by grinding up flesh with water, which was poured off, boiled and filtered; it thus became limpid and well adapted to show the slightest change in transparency which attends the development of infusoria. Equal quantities of the solution were poured into a given number of bottles, and these were divided into three series. Those of the *first* were set aside as criteria; to each of those of the *second* was added a drop of water containing active infusoria; to each of those the *third* was added a drop of the same fluid as in the second series, but after it had been boiled. All were placed together, having a similar exposure to light and heat. By a comparison of the results of the second and third series it will be seen whether the boiling has perceptibly affected the growth and reproduction of the infusoria; by a comparison of the results in either the second or third series with those of the first, the difference will be shown between the effect of adding boiled or unboiled infusoria on the one hand, and the simple exposure of the solution to the air without the addition of either, on the other. When an open bottle was used it was in most cases covered with a paper cap to exclude in a measure the dust from the air; this exclusion was of course incomplete, but as all the flasks were equally exposed and large numbers were used, any disturbance would be the same for all, and might therefore be left out of the account. As will be seen further on we have not depended solely on this form of the experiment.

Exp. XVI.—Eighteen bottles of the capacity of 25 c c. and an inch in diameter throughout and containing equal quantities of boiled and filtered beef juice were divided into three series of six each. All of series I were set aside as criteria; to all of series II were added from a bottle five drops of water containing large numbers of living Vibrios taken from a fluid in which flesh was macerating; to all of series III were added five drops of the same infusorial fluid after it had been boiled five minutes.

On the 2d day all of series II, to which the unboiled infusoria had been added, were turbid from the rapid multiplication of the animalcules, and an infusorial film was formed on the surface of the fluid. All of series I and III were unchanged.

On the 3d day all of I and III had begun to be turbid, and on the 4th were equally so with II.

Exp. XVII.—Thirty-three bottles prepared and arranged as in the preceding experiment were divided into three series of eleven each.

On the 2d day all of series II were turbid and had an infusorial film. The others were unchanged.

* In some of the later experiments, instead of beef juice prepared as above, a solution of Borden's "extract of beef" was used.

On the 3d day eight of series I and seven of series III had become turbid and the rest became so on or before the 6th day.

From the above experiments it is obvious that the boiling of infusoria has a marked effect, since the solutions, to which boiled infusoria were added, did not become invaded by animalcules sooner than those to which none had been added, while those to which unboiled infusoria were added, were in all cases invaded at least one day and in some, two or three days earlier.

In each of the preceding experiments the bottles were all more or less exposed to the air, for the paper covering was only a partial protection. The following modification of them was tried, for the purpose of ascertaining if there would be any difference in the time in which infusoria would appear in portions of the same solution enclosed in two series of flasks, to one of which infusoria had been added, and all of both series sealed at the temperature of the room, and afterwards boiled for the same time. By performing the experiment in this way the influence of dust from the air was eliminated, excepting in so far as it was present in the flasks at the time they were sealed. This would be the same in both series, as it was in the preceding experiments, the only difference being that the quantity would be less, and there would be none which had not been boiled.

Exp. XVIII.—Nineteen flasks of about 75 c. c. capacity, each containing about 15 c. c. of boiled and filtered beef juice were divided into two series, one of nine and the other of ten flasks. All of series I were sealed at the temperature of the room, without any addition to their contents. After a drop of water containing active infusoria had been added to each of series II, these were also sealed at the temperature of the room, and then all of both series boiled 15 minutes.

They were allowed to stand twelve days in a cold room in the winter, when no change having taken place they were removed to a room in which the temperature ranged from 50° to 70° F., day and night. Six days later, on the 18th from the beginning of the experiment, five flasks of each series had become turbid, and on the 20th all had become so. There was no obvious difference between the two series in the time required for the development of the infusoria. A longer time passed before the infusoria appeared than in the experiments with the open bottles, but this is to be attributed in part to the coldness of the room in which they were at first placed. But even in the warm room it was six days before they became turbid.

Exp. XIX.—Seven flasks containing boiled and filtered beef juice were divided into two series—I, consisting of three flasks, had added to the above fluid in each one drop of infusorial fluid, and were sealed and allowed to remain at the temperature of the room. Series II consisted of four flasks prepared as in series I, but after being sealed were boiled 30 minutes.

At the end of 18 hours two of series I had become turbid but none of II. At the 25th hour all of I were turbid and two of II. On the 3d day three of II were turbid and on the 4th all were.

Exp. XX.—A fluid was prepared containing half a gram of extract of beef to 100 c. c. of water. Twenty-one flasks containing this fluid were divided into four series.

SERIES I, consisting of three flasks which were sealed at the temperature of the room.

SERIES II, consisting of six flasks, had added to each one drop of dust fluid; this last was made by adding three or four grams of dust from the tops of shelves, to about 200 c. c. of boiled water which had cooled; the mixture was shaken, the heaviest particles of dust allowed to settle, and the rest poured off. At the end of a few days this fluid contained active Vibrios.

SERIES III, consisted of six flasks containing the same as those of series I, sealed at the temperature of the room, and then boiled 30 minutes.

SERIES IV, consisting of six flasks, contained the same fluid with the addition of one drop of dust water to each flask; all were boiled 30 minutes.

On the second day one of series I, and all those of II were turbid and had a film—III and IV were unchanged, and perfectly transparent. On the third day infusoria appeared in III and IV, but were confined to the surface, forming a granular looking film, the fluid remaining transparent.

The contents of the flasks were examined with the microscope. I and II contained Vibrois of a large size, some of them extending nearly across the field, and when free moved slowly. III and IV also contained Vibrios, but of a very minute size and moved with great rapidity.

The boiling in the above experiment was followed by a later appearance of infusoria in III and IV than in I and II. The addition of dust water to series II, hastened the development of infusoria. In series IV to which the same dust had been added, but the flask and contents subsequently boiled after being sealed, infusoria appeared one day later.

Conclusions.—The following conclusions appear to the writer to be justified by the observations and experiments recorded in this paper.

1st. In thermal waters plants belonging to the lower kinds of Algæ live in water the temperature of which in some instances rises as high as 208° F.

2d. Solutions of organic matter boiled for twenty-five minutes, and exposed only to air which had passed through iron tubes heated to redness, became the seat of infusorial life. Exps. I–V.

3d. Similar solutions contained in flasks hermetically sealed, and then immersed in boiling water for periods varying from a few minutes to four hours, also became the seat of infusorial life. The infusoria were chiefly Vibrios, Bacteriums and Monads. Exps. VI–XV.

4th. No ciliated infusoria, unless Monads are such, appeared in the experiments referred to in the above conclusions.

5th. No infusoria of any kind appeared if the boiling was prolonged beyond a period of five hours.

6. Infusoria having the faculty of locomotion lost this when exposed in water to a temperature of from 120° to 134° F. Exp. page 172.

7. If Vibrios, Bacteriums and Monads are added to a clear and limpid organic solution, this becomes turbid from their multiplication in from one to two days. If however they have been previously boiled, the solution does not become turbid, until from one to two days later, and in some of the experiments not sooner than does the same solution to which no infusoria have been added.